

## Protocol Rapid Plant Disease Test (LFD)

Prime Diagnostics lateral flow tests are quality products, designed for the on-site detection of pathogens (viruses, bacteria or fungi) causing disease symptoms in plants. To ensure optimum performance it is important to follow the instructions carefully. This document is a general guidance on how to use a Prime Diagnostics lateral flow tests.

### What Is In the Kit

The Prime Diagnostics lateral flow device (LFD) kit contains everything you need to test your plant. The LFD strip is encased in a cassette but contact with the LFD strip should be minimized: do not touch the sample application centre and membrane strip.

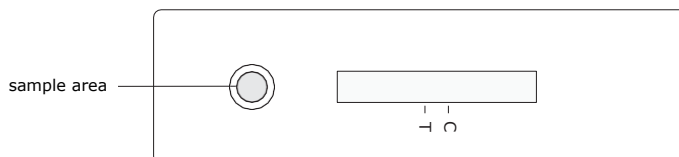
- The LFD cassettes (Fig. 1) are kept in a protective pouch for long shelf life. This pouch contains a sachet of silica gel desiccant (avoid skin contact).
- The name of the pathogen for which the test was developed is shown by label on the aluminium pouch and on the back of the LFD cassette.



Figure1. LFD Kit content; Left (top to bottom) reaction tubes with buffer and support, mini pipettes (4x), pestles (4x), Right pouches with cassettes strips (4x).

### Kit Content

- Extraction buffer and reaction tubes. The extraction buffer improves test reliability. Prime Diagnostics LFD tests will work only with the buffer supplied.
- Pestle - for grinding the plant tissue in the reaction tube.
- Foam support - to support the reaction tubes.
- Instruction for use.



- The results will become visible in a few minutes. All Prime Diagnostics LFD tests will produce a valid result within 15 minutes. Changes to the test result which happen after these 15 minutes should be ignored.
- If the test is performing well, a clear "C" line will appear. The intensity of the "T" line will vary with the amount of pathogen present in the sample.

### Short Procedure

- Collect sample material.
- Use the lid punch a hole in the sample material (Fig. 2A - C).
- Use pestle to grind sample material (Fig. 2D).
- Remove LFD cassette from the pouch (Fig. 1).
- Transfer plant sample (5 - 6 droplets) to sample application spot with the mini pipette (Fig. 2F).
- Detailed instructions are listed at page 2.

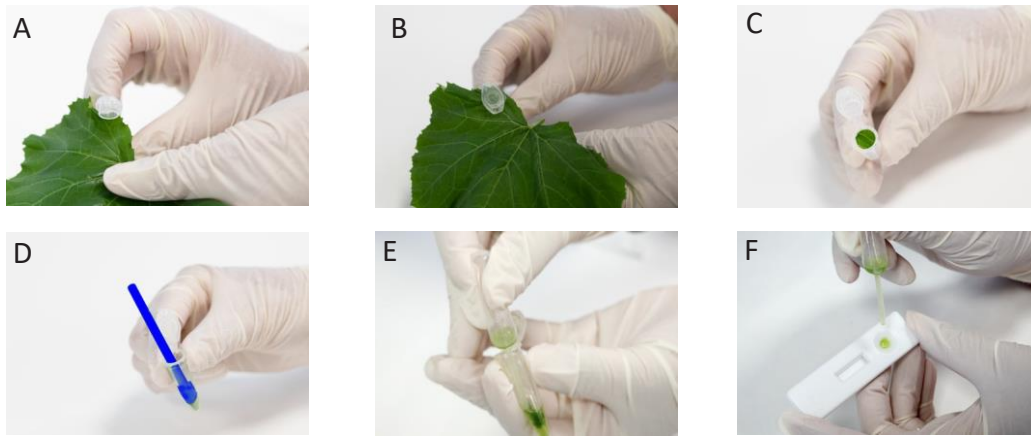
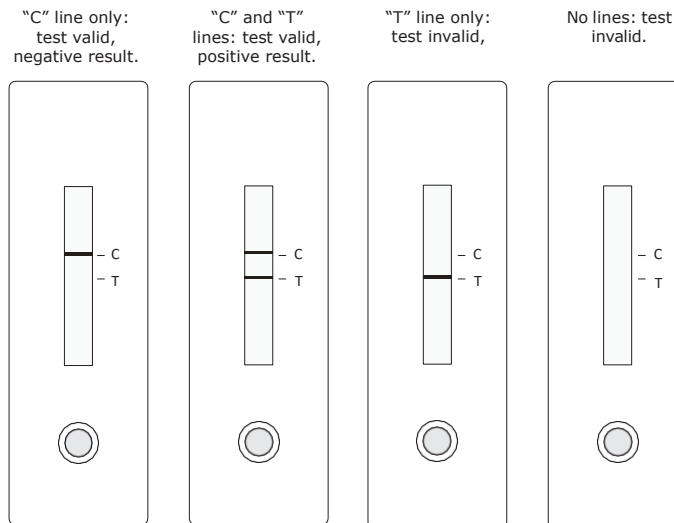


Figure 2. Workflow for performing the Prime Diagnostics lateral flow test. A. Collect plant material and open the lid of a new reaction tube containing the buffer. Next, place the reaction tube under the leaf to be sampled. B. Close the lid and remove, if needed, plant material on the outside of the reaction tube. C. Open the lid; the sampled material will become visible. D. Push the sampled leaf into the buffer and grind with a new pestle. E. Remove pestle and wait 1-5 minutes (depending on plant material). F. Transfer, with the mini pipette (5 - 6 droplets) the sample to the application area. Results will be visible within 15 minutes.

### Reading the Result



### What Does the Result Mean

A positive results indicates the sampled plant material contains the pathogen (virus, bacterium or fungus). Note that disease symptoms can be caused by mixed infections, and that further testing for other causes of disease might be necessary.

Under some circumstances, laboratory confirmation of an LFD cassette test result is recommended e.g. where a seed classification scheme is in operation, or for results of economic significance.

If the tests show positive results for quarantine organisms, the customer is responsible for complying with the local regulations to the official plant health inspection authorities.

A negative result indicates that the target pathogen was not detected in the sample. As with all diagnostic testing, a negative results does not prove that the plant is free from pathogens under test. A faint or absent "T" line may indicate a low concentration of the pathogen, uneven distribution, or recent infection. If in doubt repeat with a new LFD cassette using fresh sample, or repeat in a few days.

## Instruction For Use

| Instructions                                                                                                                                | Remarks                                                                                                                                                                                                                                                                                                                                              |
|---------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 Select plant material for testing.                                                                                                        | Do not use dead tissue, if possible select sample from symptomatic material only. Samples of suspected plant material should be broken in small pieces, added to the reaction tube with buffer. Woody material should be cut in smaller pieces using a sharp blade. Do not add too much plant material; this will cause interference with the tests. |
| 2 Open the reaction tube and punch the lid through the plant material.                                                                      | Push the plant material into the buffer before grinding.                                                                                                                                                                                                                                                                                             |
| 3 Grind the material thoroughly until no large plant parts remain.                                                                          | Cutting woody or fleshy material into smaller pieces before grinding can improve test results.                                                                                                                                                                                                                                                       |
| 4 Remove the LFD cassette from the pouch and place on a level surface.                                                                      | Open the lid slowly to avoid contamination of other materials.                                                                                                                                                                                                                                                                                       |
| 5 Transfer plant sample (5 - 6 droplets) to cassette sample application spot (Fig. 2F) using the single use pipette.                        | Transfer 5 - 6 droplets of the plant sample, using the mini pipette, to the LFD cassette's sample application spot (Fig. 2F).                                                                                                                                                                                                                        |
| 6 If the test is positive, two lines will appear. The bottom line indicating the pathogen (T) and an upper line indicating the control (C). | A faint or absent "T" line may indicate a low concentration of the pathogen, uneven distribution, or recent infection. If in doubt repeat with a new LFD using fresh sample, or repeat in a few days.                                                                                                                                                |

## Troubleshooting

| Problem                                           | Cause - Remedy                                                                                                                                                                                                           |
|---------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| No green or brown color in the extraction buffer. | Insufficient grinding, plant sample too hard, dead tissue. Try fresh sample, grind better.                                                                                                                               |
| Test runs very slowly.                            | Buffer uptake obstructed or not enough sample added. Check sample volume and, if needed add more sample using the mini pipette.                                                                                          |
| Green lines visible.                              | Green lines are a result of concentrated plant sample, and cannot be read as a valid result. Use the correct amount of sample or dilute sample.                                                                          |
| Faint lines.                                      | Low pathogen concentration; uneven distribution; sample not grinded correctly.                                                                                                                                           |
| "T" visible, but no "C" line.                     | Possible due to high level of pathogen in the sample, preventing test from working properly. Dilute one drop of the original sample into a new reaction tube containing fresh buffer and use another fresh LFD cassette. |
| No "T" line, no "C" line.                         | No buffer uptake or no good contact with buffer. Check buffer volume and adjust LFD position if appropriate.                                                                                                             |
| Damaged test kit components..                     | Please contact us at: <a href="mailto:primediagnosics@wur.nl">primediagnosics@wur.nl</a>                                                                                                                                 |